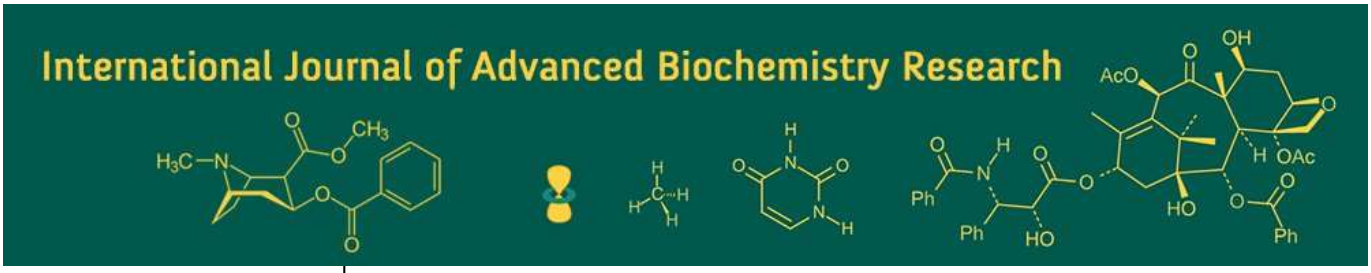

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Determination of risk factors associated with *R. equi* infection in foals

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Abstract
Rhodococcus equi infection is considered as the most common cause of severe pneumonia in foals and has a substantial impact on equine breeding farms because of its high prevalence and case fatality rate. The present study was conducted to identify the risk factor associated with *Rhodococcus equi* infection in foals. Fecal swab samples were grown on *Rhodococcus equi* specific CAZ-NB media. Based on PCR results 100 foals screened from north western of Rajasthan, 18 foals were found positive for the *Rhodococcus equi* infection as these samples showed the amplification of Vap A and Vap C genes. It was found that foals below 6 months of age, born in July and August months reared at the equine farm, having large horse population, reared on sandy soil were at higher risk for getting *R. equi* infection.

Keywords: Pneumonia, *Rhodococcus equi*, foals, Vap A, Vap C

1. Introduction
Rhodococcus equi is a Gram positive facultative intracellular coccobacillus in the order Actinomycetales and is considered as the most common cause of pyogranulomatous pneumonia and diarrhoea in foals [7, 10, 12, 17]. It is a soil-saprophytic, facultative, and intracellular pathogen with a worldwide distribution [26]. *Rhodococcus equi* is largely a soil inhabitant that survives and multiplies in the gastrointestinal tract, and it is found in the faecal matter of grazing animals [2, 9]. Virulence of *Rhodococcus equi* is dependent upon the ability of the organism to enter, survive and replicate in macrophages and is associated with the presence of highly immunogenic virulence associated proteins (Vap A, C, D, E, G, and H). Vap A is most important which is a surface-expressed, lipid modified protein that elicits an intense humoral response by foals [22].
R. equi is an important bacterial pathogen of foals in India [15]. Study of risk factors associated with *R. equi* in Indian equine population is necessary to control the foal mortalities. So the present study was carried out to assess the risk factors of *R. equi* infection in foals in Rajasthan using PCR amplification of Vap A and Vap C gene in faecal samples as an epidemiological investigation tool.

2. Materials and Methods
One hundred foals (below one year of age) irrespective of sex were screened for faecal shedding of *Rhodococcus equi.* These foals were screened from different stud farms located in Sikar, Hanumangarh, Sriganganagar, Jodhpur, Pali, Bikaner, Jhunjhunu and Jaisalmer districts of Rajasthan. Information regarding owner's particulars, animals details, number of horses reared by the owner, age of foal, gender of foals, floor of foaling pan, type of soil, pH of soil, moisture of soil, preventive measures like soil change, firing of pan, application of lime on floor of pan, bedding material etc., frequency of removing foal faeces in a day, distance of dumping of faeces from pan and clinical signs of *Rhodococcus equi* pneumonia if any were recorded. The risk factors associated with *Rhodococcus equi* pneumonia were recorded on a pre-designed questionnaire.
Faecal swab samples of 100 foals up to one year of age (irrespective of sex) were collected (In triplicate) directly from the rectum of foals using sterile swabs from various stud farms. These samples were placed in *Rhodococcus equi* specific CAZ-NB Medium and incubated at 37°C for 72 hours under aerobic conditions for culture. *Rhodococcus equi* specific virulence associated genes Vap A gene 550 base pair (bp) and Vap C gene 700 base pair (bp) nucleotide sequence present on the virulent plasmid was targeted for amplification [4].

The sequence of the primer pair used was as follows
For Vap A gene
Forward primer: 5'ACAAGACGGTTTCTAAGGCG3'
Reverse Primer: 5' TTGTGCCAGCTACCAGAGCC3'

For Vap C gene
Forward primer: 5' GGGTCGTCCATCCAAATCGA3'
Reverse Primer: 5'GGTCAGGCCTATCACCCTTG3'
The expected product size for Vap A and Vap C genes were 550 and 700 base pair (bp) respectively.

Statistical method: Simple T test was used to compare the data.

3. Results and Discussions

3.1 Age of foals

Foals found positive for faecal shedding of *R. equi* ranged one month to eleven months of age. Out of 18 positive samples, 12 foals were below six months of age and 6 foals were above six months of age.

3.2 Foaling month/Season

In the present study, it was observed that the highest incidences of *R. equi* were found in the foals born in the month of July 62.5 per cent followed by 33 per cent in October, 30 per cent in August, 22per cent in November, 16.67 per cent in June, 14.28 per cent in September, 12.5 per cent in April and 14 per cent in May. *R. equi* positive foals born in months of February, March, April, May and June were 0,0,12.5,07 and 16.66 per cent, respectively. While in the second half of the year *R. equi* shedding in the foals born in months of July, August, September, October and November were 62.5, 30, 14.2, 33 and 22 per cent, respectively (Fig: 1).

The period immediately after birth is the time at which the point-source exposure to virulent *R. equi* occurs [13]. So we have taken foaling month/season as an important risk factor for *R. equi* infection in foals in the present study, carried out in the subtropical desert climatic conditions of Rajasthan. In this area summer season is quite longer than winters and sand storms blow throughout the summer and rainy season especially in the months of June and July frequency of sand storms remains very high. Horses are long day breeder and in the studied areas breeding season starts at the end of February and continue till the end of November. However, exceptions are there and we found some foals born in December and January also. For comparing the breeding season first half of the foaling season was compared (from February to June with second half from July to November) and it was found that in the second half of the year; foals were significantly more positive for *R. equi* shedding than the first half of the year (Table : 1).

Before the start of the breeding season, there is a practice of replacing old soil with fresh soil in all the farms covered under the present study. This might be one of the reasons of low *R. equi* shedding in the foals born in February and March, later on, the soil might get infected from the mares and foals might have got an infection in April, May and June. However, faecal shedding in the April, May and June was observed lower than the foal born in July, August and October November. This might be due to very high environmental temperature (40 to 50 ˚C) in April to June in this area which may hamper the growth [27] of *R. equi*. The environmental temperature in July to November usually remains 30 ˚C to 38 ˚C and most favourable for the growth of *R. equi* [23] and it is equal to the summer season of previously reported temperate countries [5]. In the subtropical climatic condition of the area under present study, July has considered most windy and fast wind continues till September [14]. In sandy areas, barn dust is an important source of infection [11]. We observed further low faecal shedding in the foals in September, which also coincides with the second soil change which farmers usually do after the end of the monsoon season. Again rise in *R. equi* positive foals born in October and November may be due to reinfection of soil and favourable environmental temperature.

It has also been reported that infection of *R. equi* in foals also related to their immune status and the newborn foals get passive immunity by the colostrum [16]. So the immune status of the mares may also play an important role. Feed, fodder and environment play important role in the mare's immunity.

Table 1: Foaling month and prevalence of *R. equi* in foals

1st half of breeding season		2nd half of breeding season	
Month	**Prevalence in per cent**	**Month**	**Prevalence in per cent**
February	0	July	62.5
March	0	August	30
April	12.5	September	14.2
May	7	October	33
June	16.66	November	22
Mean±SE	7.23±2.96		32.34±7.35*

*$P<0.05$

The organism of *R. equi* inhabits in soil [19, 23] and temperatures 30 ˚C to 38 ˚C [23], so probably avoiding breeding in the peak rainy season may have benefited the farmer. Pyogranulomatous pneumonia due to *R. equi* is a slowly progressive disease and due to higher respiratory compensatory mechanism of horses usually, the clinical signs appear in the very late stage of the disease and many foals die even after treatment. It is reported that even after clinical recovery permanent loss to the athletic capacity occurs for forever and these foals after growing up cannot perform up to the mark in the athletic events [1].

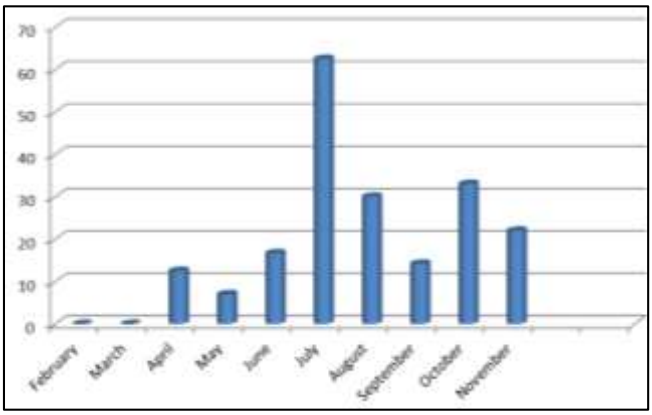

Fig 1: Foaling Month and prevalence of *R. equi*.

3.3 Type of soil

The number of foals reared on alluvial soil was 37, in desert soil 27 and dunes and associated type of soil were 36. In these foals 2, 4, and 12 foals were found positive in alluvial soil, desert soil and dunes soil, respectively. The per cent of *R. equi* positive foals in the alluvial soil, desert soil and dunes soil were 5.4 per cent, 14.80 per cent and 33.33 per cent, respectively (Table: 2, Fig: 2). In the present study, the farms where the practice of soil change was not used or used once a

year 43 % foals were found positive for *R. equi* faecal shedding, while at the farms where soil change was twice or more than twice per year 8.3% foals were found positive for *R. equi* faecal shedding.

All the stud farms included in the present study for the presence of *R. equi* in the faecal sample of foals had the pH range of 7 to 7.6 and moisture of soil about 10 to 30 per cent. We don't found any relationship between the presence of *R. equi* and soil pH and soil moisture of the pan.

Most significant factor associated with risk of *R. equi* pneumonia is the level of virulent *R. equi* in the foal's environment, specifically the concentration in soil and aerosolized dust [2, 8, 11, 12, 20, 21, 23, 24].

Table 2: Prevalence of *Rhodococcus equi* on the basis of soil type

Soil Type	Total Number of foals	Number of positive foals	% of Positive foals
Alluvial soil	37	2	5.4
Desert soil	27	4	14.8
Dunes and Associated soil	36	12	33.33

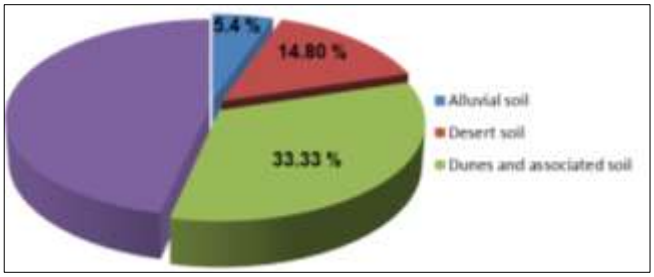

Fig 2: Prevalence of *Rhodococcus equi* reared on different type of soil.

Results obtained in this study depicts that foals reared on desert and dunes soils are at higher risk to get *R. equi* infection than the foals reared on alluvial soils. It may be because of the shifting nature of soils of the desert and dunes which increases the risk of inhalation by the foals. Inhalation of the *R. equi* bacteria through dust and sand is an important way of infection [12]. The desert and sandy area taken in this study are also affected by the regular sand storms and fast winds which allows the sand to be inhaled by the foals. That suggests the dusty environment increases the risk of *Rhodococcus equi* infection.

It is reported that the highest numbers of *R. equi* were found in surface soil, whereas almost no bacteria were found in soil observed 30 cm or more underground [25]. Surface soil harbours more number of virulent *R. equi* and removal of the upper layer of soil of stable removes the *R. equi* present in the upper layer of soil as the upper layer of soil is highly contaminated with the faeces of a foal which increases the number of virulent *Rhodococcus equi* in the soil, which is in agreement with Barton and Hughes (1984) [2] that faeces of foals is an important source of infection for the paddock. Prescott *et al.* (1984) [21] pointed out the danger of progressive development of infection in affected soil on horse farms with prolonged use because conditions favourable to the proliferation of *R. equi* were provided in the environment of a horse-breeding farm which had been established for many years.

3.4 Population density of animals (horses/mares/foals) in stable

In the present study owner of all the 18 positive cases were found in the stud farms having 15 or more horses. The stud farms having more number of horses, mare and number of foaling per year were found at higher risk of getting *Rhodococcus equi* infection of foals.

Foals exhale substantially higher concentrations of virulent *R. equi* in their breath than dust derived aerosols, suggesting the potential for an alternative contagious method of transmission of infection [18]. Chances of *R. equi* pneumonia increases with increased foal numbers and stocking density [3]. Farm-level studies indicate that the number of horses and density of mares and foals per acre seems to be positively correlated with the incidence of *R. equi* pneumonia [3, 6, 8]. Present findings support the previous reports that the incidence of the *R. equi* is related to the number of breeding mares and foals at the farm. The high number of animals and faecal shedding continuously contaminate the soil of the barn. Frequent foaling and rearing of the foal in same pan and practice of foaling line may also increase the probability of *R. equi* infection in the foals. Pans of these foaling lines rear foals of susceptible age round the year and increase the chances of heavy *R. equi* population in the soil. In the present study the highest prevalence of *R. equi* in the foals were observed in studs where the practice of foaling line was followed.

3.5 Disposal of foal's dung

In the present study the farms where disposal of foal dung was done once in a day, 43 per cent foals were found to be positive for *R. equi* faecal shedding, while at the farms where the disposal was twice or more than twice in a day, 8.3 per cent foals were found to be positive for *R. equi* faecal shedding. This was in agreement with the findings of Barton and Hughes (1984) [2] that faeces of foals is an important source of infection.

3.6 Application of lime

In the stud farms taken in this study, all were practising of application of lime in the foaling pan, so no relationship was observed in the presence of *R. equi* and application of lime.

3.7 Floor of pan

All the stud farms taken in this study were having the kaccha floor, so no relationship was observed in the presence of *R. equi* and floor of foaling pan.

4. Conclusions

Based on the present study, it can be said that foaling, foal population at the farm, age of foals, type of soil is the main risk factors for the incidences of *R. equi* in foals.

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6. Conflict of Interest: The authors declare no conflict of interest.

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